

# Expression of lymphocyte homing and adhesion molecules during intramammary infection of cows with *Serratia marcescens* or *Streptococcus uberis*: Correlation with bacterial colonization and clinical signs

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## Abstract

We wished to determine the expression of trafficking/adhesion molecules on the surface of lymphocytes isolated from infected mammary glands of cows challenged with either *Serratia marcescens* or *Staphylococcus uberis*. Healthy Holstein cows in mid lactation were infected by intramammary infusion with *S. marcescens* or *S. uberis*. Following infection, milk samples were collected at various time points. Body temperatures of the cows were taken, and milk was analyzed for colony forming units (CFU) of bacteria and somatic cell counts (SCC). Leukocytes were isolated from the milk and analyzed by flow cytometry. Percentages and types of lymphocytes were determined as well as expression of CD62L, CD11a, LPAM-1 and CD44 on these cells. We found that the percentage of lymphocytes expressing either CD62L or CD11a showed a marked increase 12 h post infection (PI) with *S. marcescens* that was not seen in cows infected with *S. uberis*. Conversely, the percentage of lymphocytes expressing CD44 increased in cows infected with *S. uberis* at 12 h PI, but the increase was not seen in cows infected with *S. marcescens*. Expression of LPAM-1 was low at all time points in both groups of cows. Body temperatures became elevated in both groups of cows, peaking at 24 h PI in *S. marcescens*-infected cows and dropping thereafter. In contrast, temperatures of *S. uberis*-infected cows continued to rise and were still elevated 96 h PI. CFU of bacteria isolated from mammary glands of *S. marcescens*-infected cows dropped precipitously 24 h PI but continued at high levels in *S. uberis*-infected cows. SCC began falling in *S. marcescens*-infected cows 48 h PI but continued to increase in *S. uberis*-infected cows. Thus, a greater percentage of lymphocytes in milk had a phenotype consistent with recruitment from the peripheral pool following infection with *S.*

**Abbreviations:** LCA, leukocyte common antigen; LFA, lymphocyte function associated antigen; LPAM-1, lymphocyte-Peyer's patch adhesion molecule; PE, R-phycoerythrin

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*marcescens* than was seen following infection with *S. uberis*. Concurrent with the increases seen in percentages of this lymphocyte phenotype, clinical signs lessened in the *S. marcescens*-infected cows.

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**Keywords:** Bovine; Adhesion molecules; Homing receptors; Lymphocytes; *Serratia*; *Streptococcus*

*Serratia* spp. are Gram-negative bacteria that have been implicated in bovine mastitis and are estimated to account for approximately 9–12% of all naturally acquired Gram-negative bacterial infections (Howell, 1972; Todhunter et al., 1991a). Of those species of *Serratia* that cause mastitis, *S. marcescens* is the most prevalent (Todhunter et al., 1991a). *Serratia* spp. have been isolated from water, soil, feed, and bedding materials (Yu, 1979), and outbreaks of *Serratia* have been attributed to contaminated teat dips (van Damme, 1982). Cows with *Serratia* intramammary infections (IMI) typically display mild clinical symptoms with the sub clinical form of infection being more characteristic than for IMI's caused by other Gram-negative bacteria (Todhunter et al., 1991b). *Serratia* IMI also tend to become chronic with a mean duration of infection lasting >160 days. The mild clinical symptoms seen during *Serratia* IMI, as well as the low numbers of bacteria shed during infection may make it difficult to identify *Serratia* as the causative agent of mastitis during outbreaks (Barnum et al., 1958). Many isolates of *Serratia* from cases of mastitis have been reported to be resistant to most approved antibiotics (Barnum et al., 1958; van Damme, 1982).

Among the Gram-positive organisms that cause mastitis, *Streptococcus uberis* is the most prevalent (Watts, 1988). In one study, 12–16% of all IMI were attributed to *S. uberis* (Jayarao et al., 1999). *Streptococcus uberis* has been recovered from soil, bedding materials, feces, and various anatomical regions of the cow (Bramley, 1982; Bramley, 1984; Jayarao et al., 1999). Similar to *S. marcescens* infections, IMI caused by *S. uberis* are often sub clinical and can persist for long periods of time in a chronic state (King, 1981; Jayarao et al., 1999; Phuektes et al., 2001).

The response to infection in the mammary gland, similar to that seen in other tissues, is dependent on the recruitment of leukocytes. The extravasation of these leukocytes is controlled by interactions between homing receptors and adhesion molecules on the leukocyte, and their cognate molecules on vascular

endothelium. Among these, CD62L is a peripheral homing receptor that has been characterized in mice, humans (Gallatin et al., 1983; Lasky et al., 1989; Siegelman and Weissman, 1989) and cattle (Bosworth and Harp, 1992; Walcheck et al., 1992; Bosworth et al., 1993). The  $\beta_2$ -integrins are a family of adhesion molecules that mediate firm attachment of lymphocytes to endothelial cells following the initial rolling and tethering step (Kurzinger et al., 1981; Arnaout et al., 1988; Springer, 1994; Salmi and Jalkanen, 1997). One of the  $\alpha$  chains in this group of molecules, CD11a, binds to a  $\beta$  chain, CD18, to form LFA-1, which is known to have a critical role in recruitment of both lymphocytes and neutrophils (Kurzinger et al., 1981; Arnaout et al., 1988; Springer, 1994; Salmi and Jalkanen, 1997). LPAM-1 is another of the integrin family of molecules consisting of an  $\alpha_4$  and a  $\beta_7$  chain. This molecule has been characterized as a mucosal homing receptor for lymphocytes (Holzmann et al., 1989; Holzmann and Weismann, 1989; Hu et al., 1992). CD44 is a proteoglycan that has been proposed to play a role in leukocyte trafficking to extra lymphoid sites of inflammation or as a nonspecific accessory adhesion molecule (Haynes et al., 1989; Aruffo et al., 1990; Bosworth et al., 1990; Denning et al., 1990; Koopman et al., 1990; Miyake et al., 1990).

In the present study, we compared the expression of CD62L, CD11a, LPAM-1, and CD44 on lymphocytes from infected quarters of mammary glands of cows infected with either *S. marcescens* or *S. uberis*. We wished to determine whether there would be differences in the phenotypes of these lymphocytes that would suggest a peripheral versus mucosal origin (CD62L versus LPAM-1 expression) or differences in adhesion molecule expression (CD11a and CD44) suggesting enhanced migratory/homing ability.

## 1. Materials and methods

Fifteen healthy, mid-lactating Holstein cows were selected on the basis of milk somatic cell counts (SCC)

of <200,000 cells/ml and the absence of detectable bacterial growth from aseptically collected milk samples plated on blood agar plates. All cows were milked twice daily, and as an indication of clinical response to infection, rectal temperatures were taken immediately prior to and at various time points following intramammary infusion of bacteria. The *S. marcescens* study was performed first, and some of the cows in this study were used again 2 months later in the *S. uberis* study. Cows were given a rigorous course of antibiotics at the conclusion of the first study, and cultured repeatedly for evidence of residual infection. Only cows with no evidence of chronic infection were used in the second study. The use and care of all animals in this study was approved by the National Animal Disease Center's Animal Care and Use Committee.

Frozen aliquots of either *S. marcescens* (gift of Dr. K. Larry Smith; Ohio State University, Wooster, OH) or *S. uberis* strain 0140 (gift of Dr. A.J. Bramley, Institute for Animal Health, Compton Laboratory, Newbury, UK) were used for challenge inoculation of cows. Prior to challenge, representative aliquots were thawed, serially diluted, and spread on blood agar plates. Following an overnight incubation at 37 °C, the concentrations of the frozen stock aliquots were calculated. For preparation of the inoculum used for intramammary infusion, aliquots were diluted in PBS to yield a final approximate concentration of 100 CFU/ml. Immediately following the morning milking, cows were infused with 2 ml of either *S. marcescens* (first study) or *S. uberis* (second study) inoculum. The contra lateral quarter of each infected quarter was infused with 2 ml of PBS. Post-plating of the final prepared inoculum confirmed that cows received 260 or 220 CFU/quarter of *S. marcescens* or

*S. uberis*, respectively. Following challenge, aseptic milk samples were collected from all infused quarters at various time points, serially diluted, and plated on blood agar plates. Following 16 h incubation at 37 °C, CFU/ml was determined. Colonies were initially identified as either *S. marcescens* or *S. uberis* based on morphological characteristics. Further biochemical tests and gas chromatography were performed by the Maryland Department of Agriculture Animal Health Section (College Park, MD) to confirm initial identification.

To quantitate somatic cells, milk samples were heated to 60 °C and subsequently maintained at 40 °C until counting on an automated cell counter (Fosso-matic model 90, Foss Food Technology, Hillerød, Denmark) as previously described (Miller et al., 1986).

Milk lymphocytes were prepared by collecting 50–150 ml of milk from infected quarters of each cow just prior to and 12, 24, 48, 72, and 96 h following experimental infection with bacteria. Raw milk was centrifuged at 400 × *g* for 15 min and the cream layer was aspirated down to a total volume of 15 ml. The sample was vortexed, then diluted with 35 ml of PBS. The sample was then centrifuged as before and aspirated to the pellet. The cell pellet was suspended in 2–3 ml of PBS and 75 µl aliquots were added to 96-well micro titer plates.

Milk cells were labeled with anti-CD45 in all wells; further characterized by labeling with appropriate antibodies (Table 1) in order to identify lymphocyte subsets, and then finally categorized for the expression of homing/adhesion molecules by 3 color fluorescence flow cytometry (Harp et al., 2004b). In brief, 50 µl of the appropriate primary antibodies were allocated to wells; plates were incubated at room temperature for 15 min, then centrifuged at 400 × *g* for 2 min and

Table 1  
Antibodies used for characterization of bovine lymphocytes

Antibody	Isotype	Specificity	Clone	Source
Mouse anti-bovine CD4	IgM	T helper	GC50A1	VMRD, Inc. Pullman, WA
Mouse anti-bovine CD8	IgM	T cytotoxic/suppressor	BAQ111A	VMRD, Inc. Pullman, WA
Mouse anti-bovine γδ TCR	IgM	γδ T cell	CACT61A	VMRD, Inc. Pullman, WA
Mouse anti-bovine B cell	IgM	B cell	VPM30	Serotec, Inc. Raleigh, NC
Mouse anti-bovine CD62L	IgG1	L-selectin	BAQ92A	VMRD, Inc. Pullman, WA
Rat anti-mouse α <sub>4</sub> β <sub>7</sub> -PE conjugate	IgG2a	LPAM-1	DATK32	BD/Pharmingen, San Diego, CA
Mouse anti-bovine CD11a	IgG1	LFA-1	BAT75A	VMRD, Inc. Pullman, WA
Mouse anti-bovine CD44	IgG3	Hermes	BAG40A	VMRD, Inc. Pullman, WA
Mouse anti-bovine CD45	IgG2a	LCA	CACTB51A	VMRD, Inc. Pullman, WA

decanted. One hundred microlitres of appropriate secondary antibodies were added and cells were incubated as before, in the dark. All plates were then centrifuged, decanted and cells were resuspended in 100  $\mu$ l of BD-FACSLyse and stored at 4 °C until data acquisition on an LSR flow cytometer (Becton-Dickinson, San Jose, CA). Data were analyzed using FlowJo software (TreeStar, Inc., Ashland, OR). Lymphocytes were gated on the CD45+ population, and then gated on the mononuclear cell fraction. Data for lymphocyte subsets are presented as the percentage of the total mononuclear cell population expressing each of the subset markers. Data for homing/adhesion molecules are presented as the percentage of each lymphocyte subset co-expressing the indicated adhesion molecule.

Significance of differences for each parameter between values for cows infected with *S. marcescens* compared to cows infected with *S. uberis* was determined by comparing the means  $\pm$  S.E.M. of data from all time points by one-way analysis of variance and Tukey–Kramer multiple comparisons test, or by Kruskal–Wallis non parametric ANOVA and Dunn's multiple comparisons tests.

## 2. Results

Table 2 shows the clinical responses of cows following infection. Body temperature decreased in

the *S. marcescens*-infected cows beginning 48 h PI but continued to increase in the *S. uberis*-infected cows. Temperatures of *S. marcescens* infected cows were significantly ( $P < 0.05$ ) higher than at time 0 only at 24 h PI. Temperatures of *S. uberis*-infected cows were significantly higher than at time 0 at 48 h ( $P < 0.05$ ), 72, and 96 h ( $P < 0.001$ ) PI. In addition, temperatures of *S. marcescens*-infected cows were significantly higher than those of *S. uberis*-infected cows at 24 h ( $P < 0.05$ ) and significantly lower at 48 h ( $P < 0.05$ ), 72, and 96 h ( $P < 0.001$ ) PI. SCC decreased 72 h PI in *S. marcescens*-infected cows, but remained at high levels in *S. uberis*-infected cows. SCC of *S. marcescens* infected cows were significantly ( $P < 0.01$ ) higher than at time 0 at 24 and 48 h PI. SCC of *S. uberis*-infected cows were significantly ( $P < 0.001$ ) higher than at time 0 at 48, 72, and 96 h PI. In addition, SCC of *S. marcescens*-infected cows were significantly ( $P < 0.05$ ) higher than those of *S. uberis*-infected cows at 24 h PI. Bacterial counts decreased beginning 24 h PI with *S. marcescens*, but continued to increase through the course of the study in *S. uberis*-infected cows. Bacterial counts in cows infected with *S. marcescens* were significantly ( $P < 0.01$ ) higher than at time 0 only at 12 h PI. Bacterial counts in cows infected with *S. uberis* were significantly ( $P < 0.001$ ) higher than at time 0 at 24, 48, 72, and 96 h PI.

Twelve hour PI, 67% of lymphocytes in milk from infected quarters of *S. marcescens*-infected cows

Table 2  
Clinical responses of cows to infection

Hours PI <sup>a</sup>	Temperature (F)		SCC <sup>b</sup>		CFU <sup>c</sup>	
	Su <sup>d</sup>	Sm <sup>e</sup>	Su	Sm	Su	Sm
0	101.3 $\pm$ 0.1	101.6 $\pm$ 0.17	30,542 $\pm$ 15,056	35,550 $\pm$ 3408	0 $\pm$ 0	0 $\pm$ 0
12	101.1 $\pm$ 0.15	102.1 $\pm$ 0.16	15,833 $\pm$ 2850	31,350 $\pm$ 3172	180 $\pm$ 58	4300 $\pm$ 960 <sup>g</sup>
24	101.7 $\pm$ 0.23	103.1 $\pm$ 0.43 <sup>f</sup>	4.7 $\times$ 10 <sup>5</sup> $\pm$ 2.6 $\times$ 10 <sup>5</sup>	2.9 $\times$ 10 <sup>7</sup> $\pm$ 3.2 $\times$ 10 <sup>6g</sup>	1.0 $\times$ 10 <sup>6</sup> $\pm$ 4.9 $\times$ 10 <sup>5h</sup>	92 $\pm$ 36
48	102.6 $\pm$ 0.24 <sup>f</sup>	101.2 $\pm$ 0.18	3.3 $\times$ 10 <sup>7</sup> $\pm$ 7.1 $\times$ 10 <sup>6h</sup>	2.9 $\times$ 10 <sup>7</sup> $\pm$ 3.6 $\times$ 10 <sup>6g</sup>	3.8 $\times$ 10 <sup>7</sup> $\pm$ 1.4 $\times$ 10 <sup>7h</sup>	2100 $\pm$ 1834
72	103.4 $\pm$ 0.39 <sup>h</sup>	101.4 $\pm$ 0.22	3.9 $\times$ 10 <sup>7</sup> $\pm$ 6.1 $\times$ 10 <sup>6h</sup>	9.4 $\times$ 10 <sup>6</sup> $\pm$ 1.6 $\times$ 10 <sup>6</sup>	7.5 $\times$ 10 <sup>7</sup> $\pm$ 1.3 $\times$ 10 <sup>7h</sup>	283 $\pm$ 144
96	103.4 $\pm$ 0.45 <sup>h</sup>	101.5 $\pm$ 0.38	2.1 $\times$ 10 <sup>7</sup> $\pm$ 4.7 $\times$ 10 <sup>6h</sup>	1.3 $\times$ 10 <sup>7</sup> $\pm$ 5.4 $\times$ 10 <sup>6</sup>	1.7 $\times$ 10 <sup>8</sup> $\pm$ 7.6 $\times$ 10 <sup>7h</sup>	1102 $\pm$ 457

All data given as Mean  $\pm$  S.E.M.,  $n = 12$  for *S. uberis* group and  $n = 10$  for *S. marcescens* group.

<sup>a</sup> Hours post infusion.

<sup>b</sup> Somatic cell count/ml of milk.

<sup>c</sup> Colony forming units of bacteria/ml of milk.

<sup>d</sup> Cows infected with *Streptococcus uberis* (Su).

<sup>e</sup> Cows infected with *Serratia marcescens* (Sm).

<sup>f</sup> Significantly different from corresponding time 0 value ( $P < 0.05$ ).

<sup>g</sup> Significantly different from corresponding time 0 value ( $P < 0.01$ ).

<sup>h</sup> Significantly different from corresponding time 0 value ( $P < 0.001$ ).

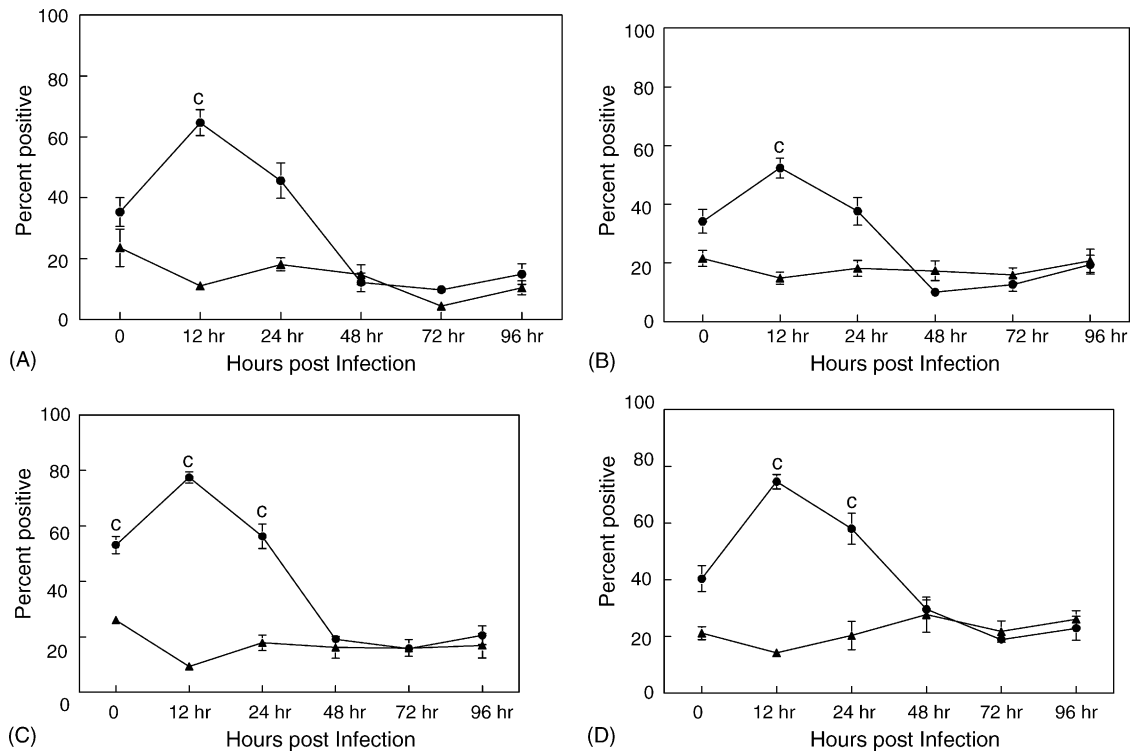


Fig. 1. Percentage of lymphocytes in milk from infected quarters co-expressing CD62L. (A) CD4+ lymphocytes, (B) CD8+ lymphocytes, (C) Gamma/delta TCR+ lymphocytes, (D) B cell marker+ lymphocytes. (●) Cells collected from cows infected with *Serratia marcescens*. (▲) Cells collected from cows infected with *Streptococcus uberis*. Statistical significance of differences between the groups at each time point is indicated by superscripts: 'a' indicates  $P < 0.05$ ; 'b' indicates  $P < 0.01$ ; 'c' indicates  $P < 0.001$ .  $n = 10$  for *S. marcescens*-infected cows and  $n = 8$  for *S. uberis*-infected cows, except for 24 and 96 h, when  $n = 7$  for *S. uberis*-infected cows.

expressed CD62L. In contrast, 12% of lymphocytes in milk from infected quarters of *S. uberis*-infected cows expressed CD62L. Percentages of CD4+/CD62L+ lymphocytes were significantly ( $P < 0.001$ ) greater in *S. marcescens*-infected cows than in *S. uberis*-infected cows 12 h PI (Fig. 1A). This was also true for CD8+/CD62L+, gamma/delta TCR+/CD62L+, and B cell marker+/CD62L+ cells (Fig. 1B, C, and 1D, respectively). Percentages of gamma/delta TCR+/CD62L+ cells were also significantly greater in *S. marcescens*-infected cows than in *S. uberis*-infected cows 0 and 24 h PI and percentages of B cell marker+/CD62L+ cells were significantly greater in *S. marcescens*-infected cows than in *S. uberis*-infected cows 24 h PI (Fig. 1C and D, respectively).

Twelve hour PI, 85% of lymphocytes in milk from infected quarters of *S. marcescens*-infected cows expressed CD11a. In contrast, 35% of lymphocytes in milk from infected quarters of *S. uberis*-infected cows

expressed CD11a. Percentages of CD4+/CD11a+ lymphocytes were significantly ( $P < 0.01$ ) greater in *S. marcescens*-infected cows than in *S. uberis*-infected cows 12 and 96 h PI (Fig. 2A). Percentages of CD8+/CD11a+ cells in *S. marcescens*-infected cows were also significantly greater than in *S. uberis*-infected cows 12 h ( $P < 0.001$ ) and 96 h ( $P < 0.05$ ) PI, while the converse was seen at 24 and 72 h PI, i.e., percentages were greater in *S. uberis*-infected cows than in *S. marcescens*-infected cows ( $P < 0.001$ ) (Fig. 2B). Percentages of gamma/delta TCR+/CD11a+ lymphocytes were significantly greater in *S. marcescens*-infected cows than in *S. uberis*-infected cows at 12 h ( $P < 0.001$ ), 72 h ( $P < 0.01$ ), and 96 h ( $P < 0.001$ ) PI (Fig. 2C). Finally, percentages of B cell marker+/CD11a+ lymphocytes were significantly greater in *S. marcescens*-infected cows than in *S. uberis*-infected cows 12 and 96 h PI ( $P < 0.001$ ) (Fig. 2D).

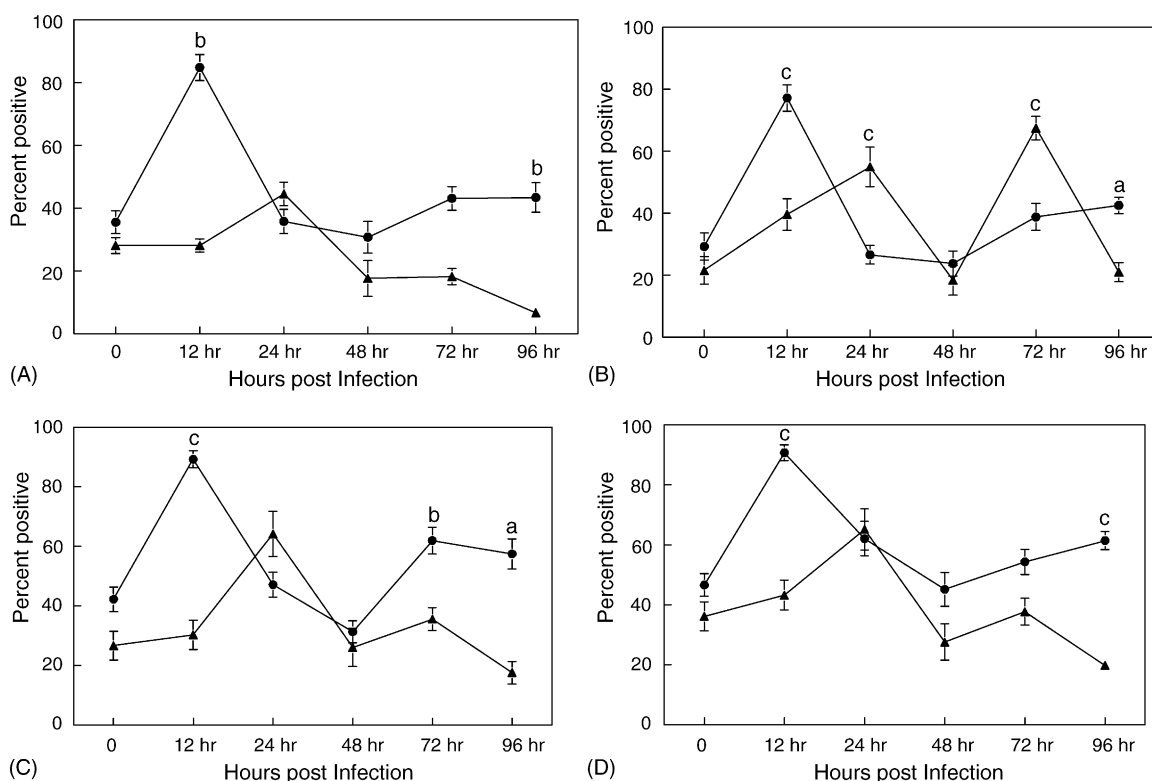


Fig. 2. Percentage of lymphocytes in milk from infected quarters co-expressing CD11a. (A) CD4+ lymphocytes, (B) CD8+ lymphocytes, (C) Gamma/delta TCR+ lymphocytes, (D) B cell marker+ lymphocytes. (●) Cells collected from cows infected with *Serratia marcescens*. (▲) Cells collected from cows infected with *Streptococcus uberis*. Statistical significance of differences between the groups at each time point is indicated by superscripts: 'a' indicates  $P < 0.05$ ; 'b' indicates  $P < 0.01$ ; 'c' indicates  $P < 0.001$ .  $n = 10$  for *S. marcescens*-infected cows and  $n = 8$  for *S. uberis*-infected cows, except for 24 and 96 h, when  $n = 7$  for *S. uberis*-infected cows.

Twelve hour PI, 19% of lymphocytes in milk from infected quarters of *S. marcescens*-infected cows expressed CD44. Conversely, 51% of lymphocytes in milk from infected quarters of *S. uberis*-infected cows expressed CD44. Percentages of CD4+/CD44+ lymphocytes were significantly greater in *S. uberis*-infected cows than in *S. marcescens*-infected cows 12, 48 h ( $P < 0.001$ ), and 72 h ( $P < 0.01$ ) PI (Fig. 3A). Percentages of CD8+/CD44+ cells in *S. uberis*-infected cows were also significantly greater than in *S. marcescens*-infected cows 0 h ( $P < 0.05$ ), 12 and 48 h ( $P < 0.001$ ) PI (Fig. 3B). Percentages of gamma/delta TCR+/CD44+ lymphocytes were significantly greater in *S. uberis*-infected cows than in *S. marcescens*-infected cows at 12 h ( $P < 0.001$ ) and 96 h ( $P < 0.01$ ) PI (Fig. 3C). Finally, percentages of B cell marker+/CD44+ lymphocytes were significantly greater in *S. uberis*-infected cows than in *S.*

*marcescens*-infected cows 12 h ( $P < 0.05$ ) and 96 h ( $P < 0.01$ ) PI (Fig. 3D).

LPAM-1 was expressed on a low percentage of all lymphocytes subsets in both groups of cows at all time points. Values ranged from 0 to 14% in *S. marcescens*-infected cows and 2–12% in *S. uberis*-infected cows.

### 3. Discussion

In previous studies, we have examined the expression of lymphocyte trafficking/adhesion molecules in milk and blood of both normal cows, and cows with Johne's disease (Harp et al., 2004a,b). In the present study, we wished to determine whether infection with either *S. marcescens* or *S. uberis*, two common causes of mastitis in dairy cattle, would result

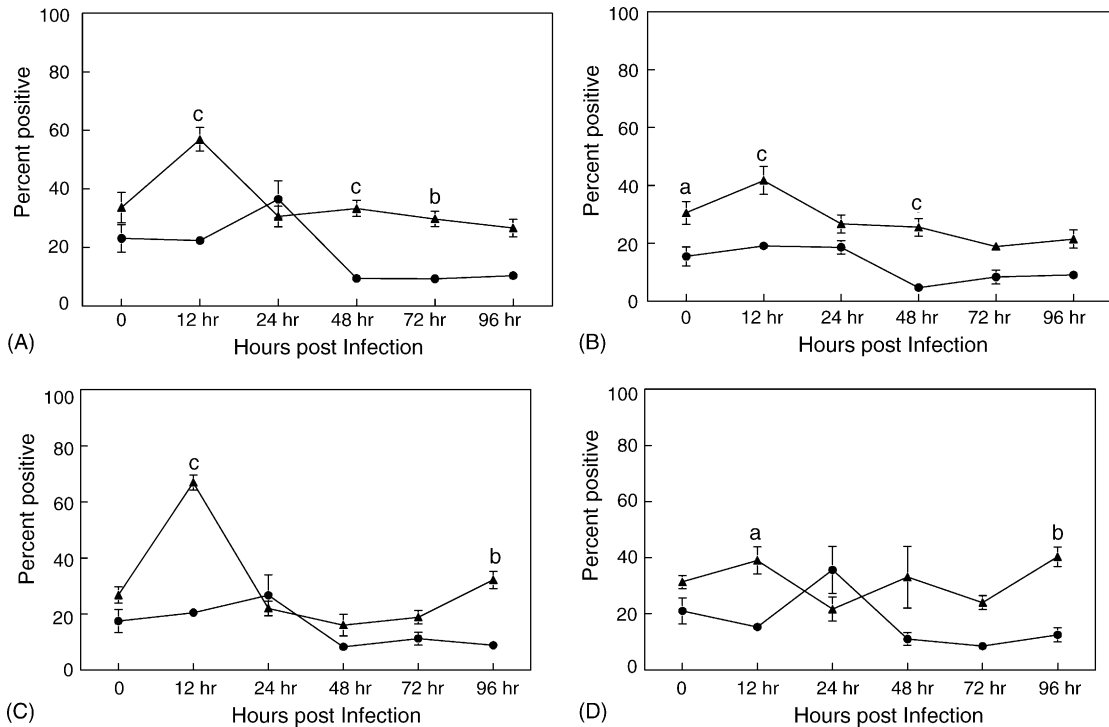


Fig. 3. Percentage of lymphocytes in milk from infected quarters co-expressing CD44. (A) CD4+ lymphocytes, (B) CD8+ lymphocytes, (C) Gamma/delta TCR+ lymphocytes, (D) B cell marker+ lymphocytes. (●) Cells collected from cows infected with *Serratia marcescens*. (▲) Cells collected from cows infected with *Streptococcus uberis*. Statistical significance of differences between the groups at each time point is indicated by superscripts: 'a' indicates  $P < 0.05$ ; 'b' indicates  $P < 0.01$ ; 'c' indicates  $P < 0.001$ .  $n = 10$  for *S. marcescens*-infected cows and  $n = 8$  for *S. uberis*-infected cows, except for 24 and 96 h, when  $n = 7$  for *S. uberis*-infected cows.

in different patterns of expression of these molecules on lymphocytes isolated from infected glands.

Cows in the present study developed clinical mastitis characterized by a febrile response, and an increase in SCC. The increase in body temperature peaked at 24 h PI in *S. marcescens*-infected cows, and SCC peaked at 48 h and declined thereafter. Bacterial counts in milk reached a mean of 4300 CFU/ml 12 h PI and declined thereafter. In marked contrast, body temperature in cows infected with *S. uberis* were highest at 72 and 96 h PI, SCC peaked at 72 h PI, and bacterial counts continued to increase throughout the study period and reached a mean  $1.7 \times 10^8$  CFU/ml 96 h PI. Thus, in *S. marcescens*-infected cows, symptoms appeared early and then declined; while bacterial counts remained relatively low. *S. uberis*-infected cows had a somewhat later onset of symptoms and bacterial counts were 5 logs higher at 96 h than those in *S. marcescens*-infected cows.

The earlier onset of symptoms in the *S. marcescens*-infected cows may be in part due to the presence

of LPS in the outer envelope of this Gram-negative bacterium. LPS is a pro-inflammatory molecule that induces a rapid innate immune response in the bovine mammary gland (Bannerman et al., 2003). Concomitant with the early appearance of fever and elevated SCC in the *S. marcescens*-infected cows, we saw a significantly higher percentage of lymphocytes expressing the CD62L and CD11a trafficking/adherence receptors in milk from infected quarters of cows infected with *S. marcescens* compared with cows infected with *S. uberis*. The high percentage of lymphocytes expressing CD62L (67% of all lymphocytes) 12 h after infection with *S. marcescens* suggests a preferential migration of cells from the peripheral lymphocyte pool into the mammary gland in response to infection. It has been previously reported that a major portion of lymphocytes in ruminant mammary gland are of peripheral origin (Harp and Moon, 1987; Harp et al., 1988, 2004a,b; Sheldrake et al., 1988; Bosworth et al., 1993). The low percentages of LPAM-

1+ lymphocytes seen in the present study is also consistent with the peripheral nature of the bovine mammary gland, since LPAM-1 has been characterized as a mucosal homing receptor (Holzmann et al., 1989; Holzmann and Weismann, 1989; Hu et al., 1992).

The high percentage of lymphocytes expressing CD11a (85% of all lymphocytes) 12 h after infection with *S. marcescens* is consistent with increased recruitment of lymphocytes into the mammary gland, because this molecule is an important accessory adhesion molecule that is necessary for efficient extravasation of lymphocytes from blood into tissue (Springer, 1994). Expression of CD62L and CD11a on lymphocytes from *S. uberis*-infected cows never reached the levels seen in *S. marcescens*-infected cows. In fact, CD62L expression remained virtually the same for the duration of the study and CD11a expression showed only a modest increase 24 h after infection (Figs. 1 and 2).

Interestingly, CD44 expression followed a different pattern from that seen with CD62L and CD11a. A higher percentage of lymphocytes expressed this molecule in *S. uberis*-infected cows than in *S. marcescens*-infected cows, most consistently and significantly at 12 h PI. CD44 has been variously characterized as playing a role in leukocyte trafficking to extra lymphoid sites of inflammation or as a nonspecific accessory adhesion molecule (Haynes et al., 1989; Aruffo et al., 1990; Bosworth et al., 1990; Denning et al., 1990; Koopman et al., 1990; Miyake et al., 1990). Since there is less evidence of inflammation 12 h PI in the *S. uberis*-infected cows than in the *S. marcescens*-infected cows, the present result is not consistent with CD44 facilitating extravasation into sites of inflammation. It is possible that CD44 may act as an accessory molecule to facilitate extravasation of a subset of lymphocytes different from those in which CD11a is up regulated.

The rapid clearance of bacteria in the *S. marcescens*-infected cows may be due in part to the growth characteristics of the bacterium compared with *S. uberis*, but may also relate to the more rapid increase in SCC in the *S. marcescens* infected cows. Milk somatic cells contain a high percentage of neutrophils, an important component of phagocytosis-mediated killing of bacteria. We saw no significant differences in the expression of CD62L and CD11a on milk neutrophils from cows infected with *S. marces-*

*cens* or *S. uberis*; however, another study of these same groups of cows reported an earlier increase in levels of IL-8, a potent neutrophil chemoattractant, and both TNF $\alpha$  and IL-1 $\beta$ , which induce a febrile response (Bannerman et al., 2004). Thus, increased percentages of lymphocytes expressing CD62L and CD11a in the mammary glands of *S. marcescens*-infected cows is consistent with the hypothesis that LPS on *S. marcescens* causes a rapid activation of resident mononuclear cells in the gland, which produce cytokines that attract peripheral lymphocytes and neutrophils to the site of inflammation, thus increasing the SCC. It is not clear from the present study what role is played by the increased percentage of lymphocytes recruited from the peripheral pool in the *S. marcescens*-infected cows. While the present study shows a temporal relationship between the presence of an increased percentage of cells expressing CD62L and CD11a, and subsequent clinical responses and bacterial clearance, proving a cause and effect relationship would require further studies utilizing cows infected with the same pathogen. By blocking or reducing the influx of CD62L+ and CD11a+ lymphocytes into the infected glands, one might determine whether these cells are directly involved in the differences in clinical signs and bacterial clearance noted in the present study.

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